numbers of vectors are available to assist the genetic manipulation and cloning of variant regulator proteins involved in secondary metabolite production. Other genetically tractable organisms could also be used for this purpose.

In a third aspect, the invention provides a method of increasing the activity of a protein that regulates secondary metabolite production comprising: (a) selecting a nucleic acid comprising a polynucleotide encoding a protein regulator of secondary metabolite production; (b) mutating the nucleic acid to create a plurality of nucleic acid molecules encoding variant regulator proteins of secondary metabolite production; and (c) selecting a variant regulator protein with more activity than the cognate, wild-type protein.

As used herein, "mutating" is used to refer to the deliberate alteration of at least one nucleotide residue of a wild-type, cognate nucleic acid sequence encoding a regulator protein of secondary metabolite production. A deliberate alteration or change in at least one nucleotide residue of a polynucleotide may be accomplished by any method known in the art. The mutation(s) can be made *in vivo* or *in vitro* and can include random, partially random or not random, *i.e.*, directed, mutagenesis techniques.

By way of non-limiting example, *in vivo* mutagenesis can be done by placing this nucleic acid molecule in a cell with a high mutation frequency, i.e. a mutagenic strain. By way of non-limiting example, Muhlrad *et al.* (*Yeast* **8**:79-82 (1992)) have developed a rapid method for localized mutagenesis of yeast genes. As a first step, the region of interest of a gene sequence is first amplified *in vitro* under error-prone polymerase chain reaction (PCR) conditions. Error-prone polymerase chain reaction (PCR) is a method of introducing amino acid changes into proteins. With this technique, mutations are deliberately introduced during the PCR reaction through the use of error-prone DNA polymerases under specific reaction conditions. With the Muhlrad *et al.* procedure, the PCR product is then co-transformed with a gapped

plasmid containing homology to both ends of the PCR product, resulting in *in vivo* recombination to repair the gap with the mutagenized DNA.

There are a variety of commercially available kits that may be used to produce mutant nucleic acid molecules by error-prone PCR (see, e.g., GeneMorph™ PCR Mutagenesis Kit (Stratagene, La Jolla, California); and Diversify™ PCR Random Mutagenesis Kit (BD Biosciences Clontech, Palo Alto, CA). Thus, a plurality of variant, *i.e.*, mutated, regulator proteins of secondary metabolite production may be produced using established mutagenesis techniques.

As used herein, the term "activity" refers to a characteristic of the regulator protein that negatively or positively affects the biological system to bring about a modulation in secondary metabolite production. By way of non-limiting example, the activity is the transcription of downstream genes involved in the biosynthetic pathway of the secondary metabolite of choice. Thus, in the present example, the phrase "more activity" refers to the property of a variant regulator protein to bring about more transcription than that effected by the cognate, wild-type regulator protein.

In certain embodiments of the third aspect, the selected variant regulator protein has more activity in a fungal cell than the cognate, wild-type protein. In certain embodiments of the third aspect, the protein regulator of secondary metabolite production is a transcription factor. In certain embodiments of the fourth aspect, the protein regulator of secondary metabolite production is a transmembrane transporter, a protein that mediates secretion, a kinase, a G-protein, a cell surface receptor, a GTPase activating protein, a guanine nucleotide exchange factor, a phosphatase, a protease, a phosphodiesterase, a bacterial protein toxin, an importin, an RNA-binding protein, an SCF complex component, an adherin, or a protein encoded within a biosynthetic cluster. . In certain other embodiments of the third

aspect, the selected variant regulator protein has more activity in a heterologous cell than the cognate, wild-type protein. In certain embodiments thereof, the heterologous cell is an organism selected from the group consisting of *S. cerevisiae, E. coli, A. nidulans, Candida sp.*, and *N. crassa*. In yet certain other embodiments of the third aspect, the selected variant regulator protein has more activity in a homologous cell than the cognate, wild-type protein. In certain embodiments thereof, the homologous cell is an organism selected from the group consisting of *Aspergillus sp., Penicillium sp., Acremonium chrysogenum, Yarrowia lipolytica, Nodulisporium sp., Fusarium sp., Monascus sp., Claviceps sp., Trichoderma sp., Tolypocladium sp., Tricotheicium sp., Fusidium sp., Emericellopsis sp., Cephalosporium sp., Cochliobolus sp., Helminthosporium sp., Agaricus brunescens, Ustilago maydis, Neurospora sp., Pestalotiopsis sp.*, and *Phaffia rhodozyma*.

In certain embodiments of the third aspect, the selected variant regulator protein has more activity in a heterologous cell and a homologous cell than the cognate, wild-type protein. In certain embodiments thereof, the heterologous cell is an organism selected from the group consisting of *S. cerevisiae, E. coli, A. nidulans, Candida sp.*, and *N. crassa* and the homologous cell is an organism selected from the group consisting of *Aspergillus sp., Penicillium sp., Acremonium chrysogenum, Yarrowia lipolytica, Nodulisporium sp., Fusarium sp., Monascus sp., Claviceps sp., Trichoderma sp., Tolypocladium sp., Tricotheicium sp., Fusidium sp., Emericellopsis sp., Cephalosporium sp., Cochliobolus sp., Helminthosporium sp., Agaricus brunescens, Ustilago maydis, Neurospora sp., Pestalotiopsis sp.* and *Phaffia rhodozyma*.

As used herein, the phrase "heterologous cell" refers to a system for gene expression, *i.e.*, an organism for gene expression, that is one other than the organism from which the selected regulator protein of secondary